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Swainsonine affects the processing of glycoproteins in vivo

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Rats, sheep and guinea pigs treated with swainsonine excrete 'high mannose' oligosaccharides in urine. The major rat and guinea pig oligosaccharide is $(Man)_5GlcNAc$, whereas sheep excrete a mixture of oligosaccharides of composition $(Man)_{2-5}GlcNAc_2$ and $(Man)_{3-5}GlcNAc$. The presence of these oligosaccharides suggests that Golgi α-D-mannosidase II as well as lysosomal α-D-mannosidase is inhibited by swainsonine resulting in storage of abnormally processed asparagine-linked glycans from glycoproteins. Altered glycoprotein processing appears to have little effect on the health of the intoxicated animal, but the accompanying lysosomal storage produces a disease state.

Swainsonine *Mannosidosis* *Oligosaccharide* *Urine* *Glycoprotein lysosomal*

1. INTRODUCTION

The plant alkaloid, swainsonine (indolizidine-1,2,8-triol), is a potent and specific inhibitor of α-D-mannosidase [1]. Prolonged ingestion of swainsonine by animals causes a lysosomal storage phenomenon with cytoplasmic vacuolation of most tissue cells, neuroaxonal degeneration and a clinical neurologic deficit typical of genetic mannosidosis [2]. This disease process can be related solely to the inhibition of lysosomal α-D-mannosidase [3].

Swainsonine also inhibits the glycoprotein processing α-D-mannosidase II in vitro [4] and in cultured cells, resulting in the synthesis of glycoproteins containing 'high mannose' and hybrid asparagine-linked glycans [5,6]. We have shown that purified swainsonine causes storage of abnormally processed mannose-rich oligosaccharides in cells in culture (unpublished).

Here, we show that swainsonine affects glycoprotein processing in vivo and we discuss the role of altered processing in swainsonine toxicosis.

2. MATERIALS AND METHODS

All chemicals used were of analytical grade obtained from BDH or Sigma of Poole (Dorset).

Weanling merino lambs, guinea pigs and Sprague-Dawley strain rats were fed ad libitum commercial feedstuffs containing 33% by wt, milled, air-dried *Swainsona canescens*. The rations contained ~8 mg swainsonine/kg. The animals were fed for 6 weeks and then returned to their normal commercial diet. During the feeding period 18 h urine samples were collected by the use of metabolism cages designed to separate urine from faeces. Urine was stored at −20°C for later use. Urinary oligosaccharides were analysed by thin-layer chromatography (TLC), gel filtration, gas-liquid chromatography (GLC) and GLC–mass spectrometry as in [8,9]. Fast-atom-bombardment (FAB) mass-spectrometry was performed as in [10].

Authentic samples of oligosaccharides isolated from the urine of human mannosidosis patients were kindly provided by Drs J.-C. Michalski and

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G. Strecker (Laboratoire de Chimie Biologique, Lille).

3. RESULTS AND DISCUSSION

3.1. *Swainsonine-induced excretion of neutral oligosaccharides*

Guinea pigs, rats and sheep fed a diet containing *Swainsona* for 6 weeks excreted large amounts of neutral oligosaccharides in their urine (fig.1). These oligosaccharides were absent or present in much lower concentrations in the urine of control animals. It was concluded that ingestion of swainsonine resulted in inhibition of lysosomal α-D-mannosidase and the accumulation in the tissues and excretion in urine of mannose-rich oligosaccharides, as in genetic mannosidosis. The pattern of excreted oligosaccharides was very similar in the guinea pig and rat. The predominant oligosaccharide (I) had the same mobility as an authentic sample of a human urinary oligosaccharide of composition $(Man)_5GlcNAc$ (M_5G). The pattern of excreted oligosaccharides was more complicated for the sheep. In particular, one of the major oligosaccharides (I) appeared to be larger, but oligosaccharides corresponding to those in the rat and guinea pig were also detected (II, III, IV). Little material corresponding to Man(α1–3)Man-(α1–4)GlcNAc (M_2G), the predominant urinary oligosaccharide in human genetic mannosidosis, was detected in any of the urines of swainsonine-treated animals. Thus the pattern of excreted oligosaccharides is different in swainsonine-induced and genetic mannosidosis.

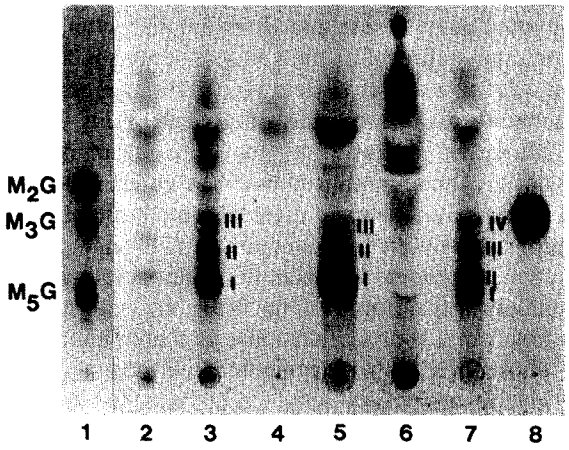

Fig.1. TLC of neutral oligosaccharides in urine of swainsonine-fed guinea pigs, rats and sheep. TLC was carried out on silica gel with *n*-propanol: H_2O (7:3, v/v) as the solvent. Carbohydrate was detected with orcinol [0.2% (w/v) in H_2SO_4 (5%, v/v, in methanol)]: (1) authentic oligosaccharides, M_2G, M_3G and isomeric mixture of M_5G; (2,3) control and swainsonine-treated rat urine; (4,5) control and swainsonine-treated guinea pig urine; (6,7) control and swainsonine-treated sheep urine; (8) M_3G.

The level of neutral oligosaccharides in sheep urine was monitored by TLC before and during ingestion of *Swainsona* and after its removal from the diet. The main oligosaccharides, I and II, appeared in the urine within 1 week of the introduction of *Swainsona* into the diet and reached a constant level within 2 weeks. This level did not change appreciably over a period of 2–6 weeks on the diet. After withdrawal of *Swainsona* from the diet, the levels of the oligosaccharides began to decrease within 2 days and the pattern returned progressively to normal over a period of 3 weeks. The larger oligosaccharides disappeared most rapidly. These observations confirmed that swainsonine induced the excretion of neutral oligosaccharides and showed that the phenomenon was reversible.

3.2. *Nature of the swainsonine-induced oligosaccharides*

The main oligosaccharides (I) in the urine of swainsonine-treated rats and guinea pigs were purified by a combination of ion-exchange, gel-filtration and preparative TLC [9]. Carbohydrate analyses were determined by GLC and GLC–mass spectrometry (table 1). Only mannose and *N*-acetylglucosamine were present in detectable quantities. The composition of rat and guinea pig I was consistent with it being $Man_5GlcNAc$. The masses of the rat and guinea pig I were determined by FAB mass-spectroscopy of mixtures of oligosaccharides I–III (fig.1), obtained by gel filtration on Bio-gel P4. FAB mass-spectrometry confirmed that the main rat and guinea pig oligosaccharide had the composition $(Man)_5GlcNAc$ and showed that rat and guinea pig components II and III had masses consistent with compositions of $(Man)_4GlcNAc$ and $(Man)_3GlcNAc$, respectively.

The main sheep urinary oligosaccharides were also purified and analysed by GLC and shown to

Table 1

Analysis of swainsonine-induced urinary oligosaccharide

	Man/GlcNAc from GLC	Molecular ions obtained by FAB mass spectrometry $m/z(M+H^+)$	Composition
Authentic oligosaccharides			
$Man_2GlcNAc$	2.15 (3)	546	–
$Man_3GlcNAc$	3.0 (3)	708	–
$Man_5GlcNAc$	5.05 (2)	1032	–
Guinea pig I	4.95 (2)	1032	$Man_5GlcNAc$
II	–	870	$Man_4GlcNAc$
III	–	708	$Man_3GlcNAc$
Rat I	5.25	1032	$Man_5GlcNAc$
II	–	870	$Man_4GlcNAc$
III	–	708	$Man_3GlcNAc$
Sheep I	2.45 (1)	1235	$Man_5GlcNAc_2$
II	2.60 (3)	1073	$Man_4GlcNAc_2$
III	1.30 (1)	911	$Man_3GlcNAc_2$
IV	1.05 (1)	749	$Man_2GlcNAc_2$

contain only mannose and *N*-acetylglucosamine. However, the Man/GlcNAc ratios suggested a higher GlcNAc content and that the separated oligosaccharides were heterogeneous. This was confirmed by FAB mass-spectrometry which showed that components I–IV corresponded to $Man_5GlcNAc_2$, $Man_4GlcNAc_2$, $Man_3/GlcNAc_2$ and $Man_2GlcNAc_2$, respectively but that smaller amounts of $Man_{3-5}GlcNAc$ were also present.

Complex glycans are normally twice as abundant as high mannose glycans in mammalian glycoproteins [11]. However, the expected storage product from the catabolism of complex glycans in the presence of a swainsonine-induced deficiency of lysosomal α-D-mannosidase, M_3G (Man(α1–3)[Man(α1–6)]Man(β1–4)GlcNAc) is not as abundant as the oligosaccharide, $Man_5GlcNAc$. The excretion of oligosaccharides containing 5 mannose residues suggests that swainsonine has blocked the conversion of high mannose asparagine-linked glycans to the complex type by inhibiting Golgi α-D-mannosidase II. Thus swainsonine-induced mannosidosis results in the accumulation and excretion of oligosaccharides derived from abnormally processed glycoproteins. The excretion by sheep of oligosaccharides containing two GlcNAc units could be due to the absence or overloading of the catabolic endohexosaminidase that catalyses the hydrolysis of the core chitobiosidic linkage. A similar deficiency of this enzymic activity has been observed in other ruminants in caprine β-mannosidosis [12] and bovine α-mannosidosis [9].

The implications of high mannose oligosaccharide storage in vivo are far reaching. The marked similarities between the morphologic and clinical expression of swainsonine intoxication and genetic mannosidosis have been noted for many years [13]. It is possible to make a direct comparison between the two forms of mannoside storage only in cattle, since in this species both forms have been reported [14,15]. Although glycoprotein processing is affected in *Swainsona* poisoning all the significant characteristics of *Swainsona* poisoning are also present in genetic lysosomal α-D-mannosidase deficiency. Even animals treated with purified swainsonine for periods of up to 120 days, apart from having a retarded growth rate and extensive tissue vacuolation, show clinical or pathological changes only in the central nervous system [16]. This suggests that the abnormal processing of glycoproteins in the presence of swainsonine has little effect on the health of the animals and that *Swainsona* toxicosis is essentially a storage disease. This conclusion is inconsistent with the assertion in [17] that

> 'the pathological effects of swainsonine are not solely attributable to its being an inhibitor of lysosomal α-D-mannosidase and are probably a consequence of abnormal processing of glycoproteins'.

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